European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

Association between IL6 gene polymorphism Via Tetra ARMs-PCR and Cancer Disease

Article History:

Name of Author:
Karrar AR Al-Salim[1], A. H. Abdul-Ameer[2], Sadiq M. J. Al-Shaikh[3], Fatimah Mohammed Saeed Mahdi[4] and Saif M. Hassan[5*]

Affiliation: *[1]Department of Medical Laboratory Technology, College of Health and Medical Technology, University of Hilla, Babylon, Iraq*
[2]Department of Anesthesia, College of Health and Medical Technology, University of Hilla, Babylon, Iraq
[3]Department of Nursing. Alzahrawi University. Karbala. Iraq.
[4]Department of pharmacology and toxicology, pharmacy college, Al-Zahraa University for Women, Karbala, Iraq.
[5]Department of Medical Laboratory Technology, College of Health and Medical Technology, University of Hilla, Babylon, Iraq.

Corresponding Author:
Karrar AR Al-Salim
(karar_abd@hilla_unc.edu.iq)

Received: 29-11-2025
Revised: 10-12-2025
Accepted: 20-01-2026
Published: 08-02-2026

Abstract: ***Background***: Cancer has emerged as a significant public health challenge, In Arab homeland the cancer disease highly occurrence. The interleukin 6 (IL6) cytokine families is crucial for various physiological functions, including inflammation, immune responses. IL6 polymorphisms rs1800795 may be a tumor marker for cancer therapy were significantly associated with the prognosis of cancer. Considering these promising results. ***Methods***: Genotyping of polymorphisms in IL6 gene was acted in Babylon province (30 cases and 30 controls). Administrative impacts of single-nucleotide polymorphism rs1800795 via ARM-PCR technique on IL6 creation in blood invigorated with a Cancer diseased item were evaluated. ***Results***: The rs1800795 polymorphism is related with expanded protection from cancer diseased case-control (ODD Ratio (OR) of risk allele GG was = 9.57, 95% CI: 1.93-47.380.771. The allele frequency of allele G was 49(81.7%) with OR= 7.17, 95% CI: 3.1-16.5, P=0.0001. ***Conclusion***: This study concluded that the GG and CG genotypes of the IL6 (rs1800795) polymorphism is a risk factor for contracting cancer disease and suggested that it plays a significant role in the severity of the disease among Babylon peoples, Iraqi.

Keywords: Cancer disease, IL6, polymorphism, SNP rs1800975.

INTRODUCTION

The Interleukin 6 (IL6) gene is responsible for encoding a cytokine that plays a crucial role in inflammation and the development of B cells which can trigger fever in individuals suffering from autoimmune disorders or infections (Jones and Jenkins, 2018, Khraibet, M. R., & Kadhim, E. J. (2025)). The activity of this gene is associated with numerous disease conditions linked to inflammation (Katsura et al.,2017,).

The IL-6cytokine family is crucial for various physiological functions, including inflammation, immune responses, and hematopoiesis (Tanaka et al.,2018). Disruption of IL6 signaling has been linked to chronic inflammation, autoimmune disorders, infectious diseases, and cancer (Rose-John et al., 2018: Jones and Jenkins, 2018), where it frequently

serves as a diagnostic or prognostic marker for disease activity and treatment response. IL6is regarded as a pivotal cytokine that connects inflammation and cancer, garnering significant interest as a potential target for therapy (Hunter and Jones, 2015).

Suggesting a role for IL6 in the regulation of T cell effector capacities, just as in the movement of tuberculosis in people (Gao, et al.,2007,).

This study aimed to detection the single-nucleotide polymorphisms (SNP) in IL6 for their relationship with cancer disease in case –control study. We tracked down that, an uncommon hereditary variety in the IL6 gene, rs1800795, are essentially connected with cancer disease in middle of Iraq and discuss our results with closed related studies in many countries through the world.

MATERIAL AND METHODS:

Study Samples:

Study samples with various clinical highlights of cancer disease were enlisted at tumor centers in Marjan Hospital, Babylon, Iraq. The sampling (blood samples) was done from of patients after 6 months of diagnosis of tumor (metachronous tumor) with age ranged from 35-70y, all clinical manifestations depended on patient history (Ng et al.,2010). Human of control group without a clinical history of tumor. Whole blood samples were collected in EDTA tube 5ml/ patient and healthy for each, Study period ranged from June- October 2023 ,30 cases with cancer and 30 Healthy human (Imran, and Ali, 2015, Kazem, A. J., Jorani, L. E., & Al-Azzawi, A. K. J. (2025)) .

Genomic DNA Extraction, SNP Selection, and Genotyping

This study commencing by collected3ml of peripheral blood of patients and healthy persons (for each). Blood samples loaded in labeled vial contain ethylenediaminetetra-acetic acid (EDTA) and kept in deep freeze (-20C°) until used. Genomic DNA was extracted by FavorPrep Genomic DNA Mini Kit (Blood/ Cultured cell) (Taiwan com.) (Al-Jubory and Imran,2020, Mohsin, M. D., & Edan, B. J. (2024)). The procedure was achieved according to the method recommended by the manufacturing company (Taiwan com.). For the exploratory associate, SNP rs1800796 was genotyped were dictated by Tetra-ARMs PCR technique.

Genotyping of cytokine polymorphisms

ARMs PCR polymorphism (ARMs-PCR) technique was followed for detection genotypes of IL-6 (rs1800795) in Cancer disease via was done utilizing four primers: Fo: GACATGCCAAAGTGCTGAGTCACTAA, Ro: GAATGAGCCTCAGACATCTCCAGTCCTA, Fn: GCACTTTTCCCCCCTAGTTGTGTCTTCCG, Rn: ATTGTGCAATGTGACGTCCTTTAGCTTG. PCR conditions: 93°C/3min.32 cycles:93°C/30Sec. annealing temp.61°C/40Sec. extension temp.72°C/35Sec. final extension 72°C/3min. cool step 4°C. 5ul of PCR product were loaded in 1.5 agarose gel pre-stained with Ethidium bromide by electrophoresis on ready in Tris-boric acid derivation EDTA (TBE), subjected PCR products for electrophoresis 50volt/ 60min. Item groups were envisioned on an UV-transilluminator and pictures were The PCR was performed under the conditions depicted by Imran et al, (2019).

Statistical analysis:

The allele and genotype frequencies were dictated by direct counting. The Chi χ2 test was utilized to analyze allele and genotype conveyance in patients with tuberculosis and sound control subjects. The Odd Ratio (ORs) and 95% confidence intervals (CIs) were determined with SPSS. The Hardy-Weinberg harmony of every polymorphism was dictated by the program HWE. Single direction examination of change with Bonferroni remedy was utilized to contrast IL-6 levels among members and various genotypes. All calculations were finished utilizing P-value 0.005.

RESULTS.

Targeted region in gene IL6 and SNP validity:

The targeted IL6and rs1800795 SNP located at 22727026 of IL6 on Chr:7 on reference strain NO. NC_000007.14. The figure 1 also shows a check for the location of the SNP rs1800795, the type of the wild allele G mutant to allele C in SNP rs1800795 (Figure 1).

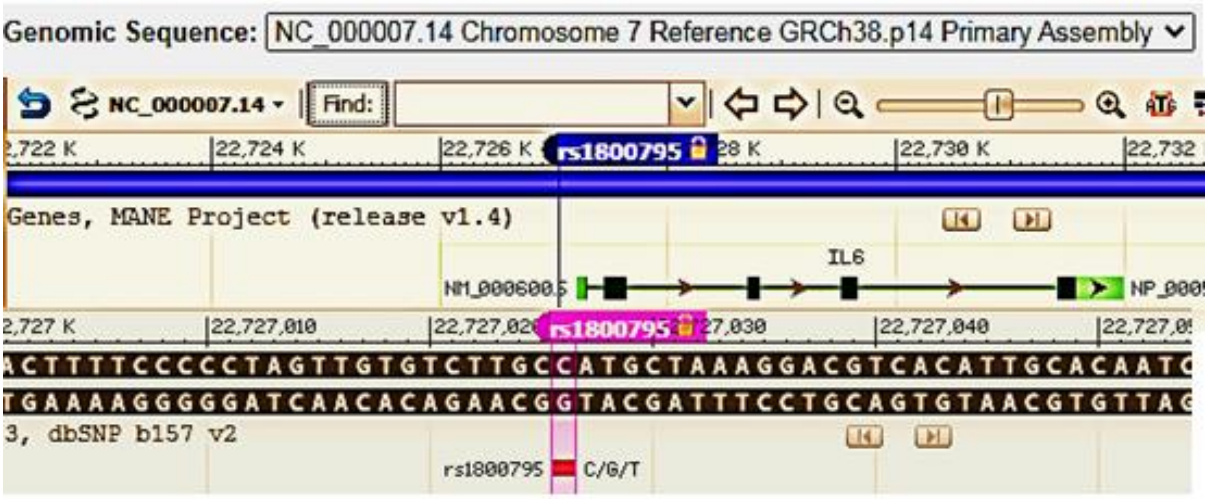

Figure 1: Illustration location rs1800795 SNP at 22727026 of IL6 gene on Chr:7 reference strain NO. NC_000007.14.

IL6 SNPrs1800976 Genotyping and allele frequency calculation based on ARM-PCR technique.
Amplification targeted SNPrs1800975 of IL6 gene via ARM-PCR technique, the four primers were successfully amplification partial sequence under interest, the wild allele CC showed two bands, main band 302bp and C allele band 206bp, while the heterozygous mutant allele CG showed three bands;302bp, 206bp and 152bp while the homozygous mutant allele show two bands, main band 302 C allele and 152bp G allele.in both patients and healthy group Figure 2-3.

Case group shown 7 genotypes of homozygous GG genotypes, 6 genotypes with CG genotypes and 3 genotypes CC.
While healthy groups shown unique genotypes: 13 wild genotypes, 4 heterozygous mutant genotypes, while only one homozygous mutant allele figure 3.

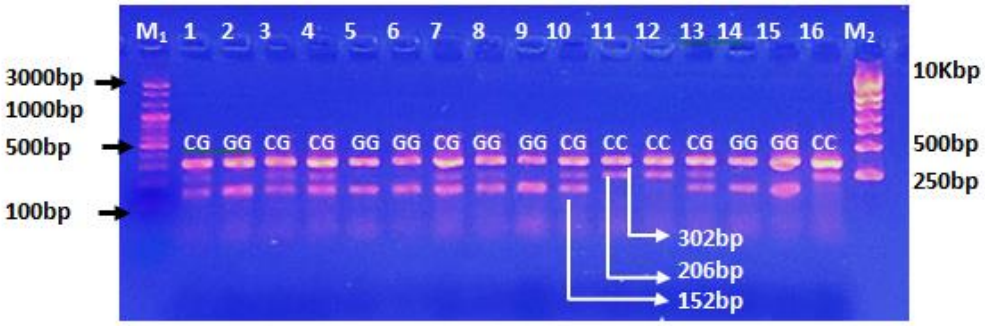

Figure (2): Gel electrophoresis of T-ARMS-PCR IL-6-174 C>G rs1800795 genotype in cancer Patients, lane 1-3-4,7,10,13 GC genotype ;2,5-6,8-9,14-15 GG genotypes;11-12 CC genotype, the PCR products were analyzed by gel electrophoresis on 1.5% of agarose , 50V for 60 min. M1= Marker 100-3000bp,M2=250-10000bp.

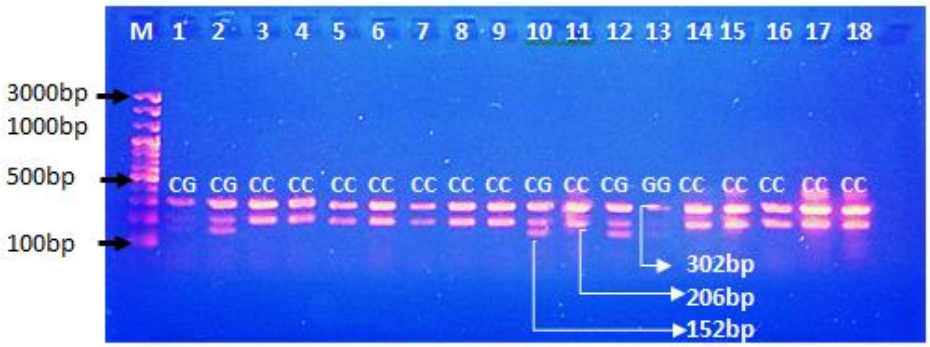

Figure (3): Gel electrophoresis of T-ARMS-PCR IL-6-174 C>G rs1800795 genotype in healthy group, lane 3-9,11,14-18 CC genotypes; 1-210,12, GC genotype ,13 GG genotype, the PCR products were analyzed by gel electrophoresis on 1.5% of agarose , 50V for 60 min. M= Marker 100-3000bp,

Genotyping and allele frequency of IL6 SNP rs1800795:

The results of this study showed that rs1800795 was significantly associated with increased cancer risk in allelic, (the homozygous mutant allele GG showed high OR value = 9.57, 95% CI:1.93-47.38, *P* = 0.257, and the heterozygous mutant allele CG with OR = 1.9 95% CI:0.62-5.8, *P* = 0.2. The allele frequency of allele C was 49(81.7%) with OR= 7.17, 95% CI: 3.1-16.5, P=0.0001 (Table 1).

Table 1: Genotypes distribution of Il-6 gene SNP: ID=rs1800795 C>G between Patients group and healthy group. Abbreviation: SNP: single nucleotide polymorphism

SNPID	Genotype	Patients N=30	Control N=30	OR(95%CI)	P-value
rs1800795 C>G	CC	6(20%)	21(60%)	reference	
	CG	11(36.6%)	7(32%)	1.9 95%CI:0.62-5.8	0.2
	GG	13(43.3%)	2(8%)	9.57 95%CI:1.93-47.38	0.257
Allele Frequency	C	23(38.3%)	37(61.6%)	0.139 95%CI:0.06-0.3	0.0001
	G	49(81.7%)	11(18.3%)	7.17 95%ci:3.1-16.5	0.0001

Abbreviations: ; OR: Odd Ratio; CI: Confidence Interval.

DISCUSSION:

Interlukine 6 gene is responsible for encoding a cytokine that plays a crucial role in inflammation, which can trigger fever in individuals suffering from autoimmune disorders or infections (Coussens, and Werb, 2002).

The most recent annual report from the Iraqi Cancer Registry, published in 2018, indicated that out of an estimated population of 38 million, there were a total of 31,502 new cancer cases. Additionally, the report recorded 10,293 cancer-related deaths. The breast cancer had the highest incidence rate among the ten most common cancers in Iraq (Annual Report Iraqi Cancer Registry 2018).

Our research indicates that the mutant homozygous (GG) and heterozygous (CG) genotypes are the predominant variant among cancer patients population under sampling in Babylon province, aligning with previous studies ((Bao et al., 2008). Their findings revealed that wild type allele CC was 23(38.3%) in patient group while 37(61.6%) in healthy one. On the other hand, GG genotype 49(81.7%) of patients while 11(18.3%) in healthy group (Table 1). This observation points to a potential association between IL6 (rs1800795 C>G) and cancer in the Babylon population. Furthermore, anthers studies demonstrated that individuals with the (rs1800795) CC genotype exhibit a protective effect against severe cancer, whereas those with the (rs1800795 C>G) GG genotype face a heightened risk of developing acute symptoms (Yang et al., 2014).

Cancer has emerged as a significant public health challenge, in Arab homeland the cancer disease highly occurrence (Arafa et al., 2020). Wang et al.,(2015) reported that by 2020, cancer will become the leading cause of illness and death in many developing countries. In the majority of Middle Eastern nations, including Qatar, Oman, Kuwait, Bahrain, Yemen, Syria, Iraq, Iran, Palestine, and Jordan, cancer ranks as the second leading cause of premature mortality, following cardiovascular diseases. However, in Lebanon, it holds the position of the primary cause of death. In Saudi Arabia and the United Arab Emirates, cancer is among the top three causes of death (Bray, et al., 2021). Over the last thirty years, the average rate of obesity in Arab nations has surged significantly, rising from 6.5% in 1975 to 20% in 2016. This trend has been linked to specific genetic polymorphisms found in the Arab population (Younes et al., 2021).

The current study found significant association between the IL-6 polymorphism (rs1800795) and the risk of cancer disease. It identified three genotypes: CC representing the wild-type genotype, CG indicating the mutant heterozygote genotype, and GG denoting the mutant homozygote genotype. These findings align with the research conducted by (Joshi et al.,2014), which concluded that variations in the IL-6 gene at the -174 G/C position may contribute to the risk factors for cancer among patients in Iraq.

To address this critical issue, it is essential to identify and manage cancer risk factors in a timely and effective manner. The causes of cancer are influenced by both genetic and environmental elements (Luo et al.,2009). Consequently, gaining insights into the role of genetic factors in cancer can aid in its prevention. Interleukin-6 (IL-6) is a well-established pro-inflammatory cytokine that plays a role in cardiovascular diseases. Increased levels of IL-6 and its primary effector have been linked to various stages of cancer progression, including initiation, promotion, malignant transformation, invasion, and metastasis (Rašková et al.,2022).

IL-6 promoter polymorphisms were significantly associated with the prognosis of cancer. Considering these promising results, IL-6 promoter including rs1800795, rs1800796 and rs1800797 may be a tumor marker for cancer therapy (Peng et al.,2018).

Numerous factors can individually or collectively lead to the development of cancer, particularly in women with a genetic susceptibility to the disease or those exposed to high-risk elements (Alrawi,2022).

Liu et al., 2017 conducted a study that revealed no significant differences in the genotype and allele frequencies of the IL-6 (rs1800795) SNP among two groups of cancer patients from the Babylon population in Iraq. Their findings indicated that both genotype and allele distributions were consistent across these groups. Another investigation found that genotyping of IL-6 rs1800795 (G/C) showed the presence of all genotypes in the entire cohort. However, the GG genotype of IL-6 rs1800795 was more prevalent in patients with cancer (52.5%) (Magalhaes et al.,2013) .In contrast to the findings of the current study, polymorphisms in the IL-6 gene at the (-174 G/C) position have been associated with an increased risk of cancer disease, suggesting that this gene may serve as a significant risk factor for the cancer among the Iraqi Arab population (Iraqi Cancer Board. Annual Report Iraqi Cancer Registry 2018). Additionally, a recent study involving the Turkish population identified a significant association between the IL-6 polymorphism at the (rs1800795) site and cancer, which contradicts the results of the present research (Arafa, and Farhat,2022). Furthermore, research by Hussein et al.,(2024) indicated that the presence of both the G allele and the GG genotype of IL-6 is strongly correlated with an increased susceptibility to cancer in Iraq.

In summary, our findings have revealed that IL-6 -174G>C (rs1800795) is a statistically significant SNP associated with susceptibility to cancer disease Babylon population. Given the constraints of our study, additional research at the cellular and molecular levels, involving larger sample sizes, is necessary to explore the potential regulatory influence of this SNP on IL-6 production and its subsequent role in the onset and progression of cancer disease.

Ethical approval

Author(s) hereby declare that all actions have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

REFERENCES:

1. Abd.Al-Kahdum S. A., Imran Z. K., and Khdhier H. M. (2019). Molecular Typing of *Malassezia* species By RFLP-PCR and Evaluate Antifungal Activities of Some Plant Extracts. *Plant* Archives, 19(Suppl.1): 217-221.
2. Khraibet, M. R., & Kadhim, E. J. (2025). Anti-Lung Cancer Activity of the Herbal Medicinal Plant Cycas revoluta. *Hilla University College Journal For Medical Science*, *3*(2), 25-30.
3. Kazem, A. J., Jorani, L. E., & Al-Azzawi, A. K. J. (2025). Concept of Marginal Fit Accuracy of a Single Crown Prosthesis Using Conventional Impression Techniques and Intra-Oral Scanner. *Hilla University College Journal For Medical Science*, *3*(2), 63-69.
4. Mohsin, M. D., & Edan, B. J. (2024). Impact of Physical Activity and Body Size on Serum Level of Anti-Müllerian Hormone in Women in

Babylon Province. *Hilla University College Journal For Medical Science*, *2*(2), 49-57.
5. AL-Jubory R.J. and Imran Z.K.(2020). Molecular Detection of Abundance of Uncultured Lavage Microbiota and Evaluated the SNPs in Partial Sequence of CARD9 Gene. The Journal of Research on the Lepidoptera: 5(2): 256-269.
6. Alrawi N.N.(2022). A review on breast cancer in Iraq and future the rapies in sights. Baghdad Journal of Biochemistry and Applied Biological Sciences 3(01):4-16.
7. Arafa, M.A., D.M. Rabah, and K.H. Farhat,(2020). Rising cancer rates in the Arab World: now is the time for action. East Mediterr Health J, 26(6): 638–640.
8. Arafa, M.A., Farhat, K.H. (2022).Why cancer incidence in the Arab counties is much lower than other parts of the world?. J Egypt Natl Canc Inst 34, 41.
9. Bao S, Yang W, Zhou S, Ye Z (2008). Relationship between single nucleotide polymorphisms in− 174G/C and− 634C/G promoter region of interleukin-6 and prostate cancer. J Huazhong Univ Sci Technolog Med Sci, 28, 693-696.
10. Bray, F., Laversanne, M., Weiderpass ,E., et al., (2021)The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer, 127(16): 3029–3030.
11. Coussens, L. M. and Werb, Z. (2002). Inflammation and cancer. Nature 420:860.
12. Gao, S. P., Mark, K. G., Leslie, K.et al. (2007). Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. J. Clin. Invest. 117:3846.
13. Hussein K. J., Saihood A.S., Sharif Y. H. (2024).Polymorphism specific Allele Frequencies on Cervical Cancer. Indones J Obstet Gynecol 2. (3) : 120-129.
14. Hunter, C.A. and Jones, S.A.(2015). IL-6 as a Keystone Cytokine in Health and Disease. Nat. Immunol. 16, 448–457.
15. Imran, Z. K. and Ali, E. K., (2015). Molecular identification of Candida glabrata and C. parapsilosis based on sequencing analysis of rDNA. Valley International. Journal., 2(12).
16. Imran,Z.K., Khdhier H.M. and Al-Kahdum S.A.A.(2019). Molecular Typing Of Malassezia species by RFLP-PCR and Evaluate antifungal activities of some plant extracts. Plant Archive,19(1): 217-221.
17. Iraqi Cancer Board. Annual Report Iraqi Cancer Registry (2018) Iraqi CancerRegistry Center, Ministry of Health and Environment (Baghdad, Iraq).
18. Jones, S.A.; Jenkins, B.J.(2018). Recent Insights into Targeting the IL-6 Cytokine Family in Inflammatory Diseases and Cancer. Nat. Rev. Immunol., 18:773–789.
19. Joshi,N., Kannan,S., Kotian, N. et al.(2014). Interleukin 6 174G>C polymorphism and cancer risk: Meta-analysis reveals a site dependent differential influence in Ancestral North Indians. Human Immunology 75: 901–908.
20. Katsura, A.; Tamura, Y.; Hokari, S.; Harada, M., et al.(2017). ZEB1-Regulated Inflammatory Phenotype in Breast Cancer Cells. Mol. Oncol. , 11: 1241–1262.
21. Luo J., Solimini N.L. and Elledge S.J. (2009). Principles of cancer therapy: oncogene and non-oncogene addiction. Cell, 136 : 823-837.
22. Liu TZ, ZQ G, Wang T, et al (2017). Meta-analysis of the role of IL-6 rs1800795 polymorphism in the susceptibility to prostate cancer: Evidence based on 17 studies. Medicine, 96, e6126.
23. Magalhaes JF, Cortinhas AJ, Albuquerque CM, et al (2013). Interleukin-6 gene -174G>C and -636G>C promoter polymorphisms and prostate cancer risk. Mol Biol Rep, 40:449-55.
24. Ng A.K., Kenney, L.B., Gilbert, E.S., Travis LB.(2010). Secondary malignancies across the age spectrum. Semin Radiat Oncol. 20:67–78.
25. Peng X, Shi J, Sun W, et al.,(2018). Genetic polymorphisms of IL-6 promoter in cancer susceptibility and prognosis: a meta-analysis. Oncotarget. 9(15):12351-12364.
26. Rašková M, Lacina L, Kejík Z, et al.,(2022). The Role of IL-6 in Cancer Cell Invasiveness and Metastasis-Overview and Therapeutic Opportunities. Cells. 21:11(22):3698
27. Rose-John, S. Interleukin-6 Family Cytokines. Cold Spring Harb. Perspect. Biol. 2018, 10, 1–18.
28. Tanaka, T.; Narazaki, M.; Kishimoto, T. Il-6 in Inflammation, Immunity, and Disease. Cold Spring Harb. Perspect. Biol. 2014, 6, 16295–16296.
29. Wang H, Naghavi M, Allen C, Barber RM, et al. GBD 2015 Mortality and Causes of Death Collaborators Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388:1459–544.
30. Yang M, Li C, Li M (2014). Association of interleukin-6 (-174 G/C) polymorphism with the prostate cancer risk: A metaanalysis. Biomed Rep, 2, 637-43.
31. Younes, S., et al., Genetic polymorphisms associated with obesity in the Arab world: a systematic review. Int J Obes (Lond), 2021. 45(9): p. 1899–1913

